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THE IMPORTANCE OF THE SPLEEN IN RESISTANCE TO INFECTION

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ALTHOUGH there is a widespread impression that the spleen plays a part in resistance to infection and statements to this effect have found their way into many text-books, very little experimental evidence has been offered to support this view or to refute it. There exists a vast literature dealing directly or indirectly with the relation of the spleen to infectious processes, but most of the work which has been done is fragmentary, conflicting, and unconvincing. Far-reaching inferences have been drawn and laws deduced from a series of three or four animals. Complete autopsies are almost entirely wanting; cultures have not been standardized; racial and individual susceptibility to different bacteria has frequently been ignored; and, in addition, the normal mortality of laboratory animals is not sufficiently taken into account. It is assumed that the experiments are performed on normal animals, whereas the contrary is frequently the case; and the conflicting results obtained are often due to the fact that some of the animals are the victims of pathologic processes that have nothing to do with the experiment. It is a matter of common observation that nearly all the animals which are commonly used in experimental work are the carriers, not only of a host of parasites, but of a number of infections, epidemic and otherwise. The parasitic worms in dogs, of which there are a great many varieties; the pneumonia, septicæmia, and coccidiosis of rabbits; the paratyphoid fever and intestinal worms of mice, the chicken and hog cholera bacilli, which are pathogenic for widely different species of animals: all these are examples only too well known to require emphasis. Under these circumstances, it becomes self-evident that no experimental work can be entirely trustworthy which does not include the recognition and elimination of these outside sources of error.

Especially in experiments dealing with the effects of bacteria and their toxins is it necessary, by very careful and complete examinations, both gross and microscopical, to exclude a mortality due to chance infection; and, in addition, blood or tissue cultures should be made, to establish, if possible, the cause of death. This involves a tremendous amount of work, but is indispensable to accurate results and sound conclusions.

Of the work hitherto done on the relation of the spleen to resistance to infection the most convincing is that of Pfeiffer and Marx.[1] These authors, working on the formation of immune bodies in cholera, found that these substances were present in the spleen and bone-marrow in much greater quantity than elsewhere in the body. This condition persisted for months until finally the bacteriolytic content of the blood equalled that of the spleen. While removal of the spleen prior to the immunizing process did not prevent the formation of immunity, it was found that if the immunization was attempted before splenectomy, immunity failed subsequently to develop. These results of Pfeiffer and Marx for cholera have been confirmed by Wassermann [2] for typhoid, and by numerous other observers.

Much experimental work has been done to test the resistance of animals to infection following removal of the spleen. It is, of course, an observation of great antiquity that the operation of splenectomy is not followed by death. Indeed, one may live for years without suffering any apparent ill effects from the absence of the organ; but this does not settle the problem as to whether or not a splenectomized person can weather a critical illness. Nor do we know how long it takes or to what extent it is possible for the bone-marrow or the lymphatic system to compensate for the loss of the spleen. Among the experiments undertaken to clear up this question are the following:

Roger,[3] who injected rabbits intravenously with a virulent culture of anthrax, found that the splenectomized animals died sometimes before, sometimes after the controls. This result might be expected, since rabbits have little or no resistance to anthrax, and the splenectomy would probably not materially hasten the process.

Bardach [4] injected twenty-five splenectomized dogs with 1 c.c. of anthrax culture, and found that of these animals, normally resistant to anthrax, nineteen died. Only five of the twenty-five normal control animals died after a similar injection. In a second series of experiments, Bardach attempted to immunize rabbits with attenuated cultures of anthrax. Of thirty-five splenectomized animals, twenty-six died of anthrax. The controls showed a much lower mortality.

Tizzoni and Cattani [5] inoculated three groups of rabbits with tetanus cultures. Those vaccinated and not splenectomized resisted the inoculation; whereas those vaccinated fifteen days after splenectomy died in the same time as the unvaccinated animals.

On the other hand, other observers who tried similar experiments

failed to find any difference between splenectomized and normal animals. Kourlow [6] inoculated splenectomized rabbits intravenously with cultures of chicken cholera, anthrax, and Staphylococcus pyogenes, and found that the spleenless animals died sometimes before, sometimes after the controls.

Martinotti and Barbacci [7] injected splenectomized rabbits with anthrax cultures, but failed to note any difference between these and the controls.

Courmont and Duffau [8] injected rabbits with pyocyaneus, staphylococcus, and streptococcus, and likewise obtained conflicting results; they concluded that the kind of organism and especially the time after splenectomy were important in determining whether splenectomy made the animal more or less resistant to infection. In these experiments the conclusions in each case were drawn from the behavior of one animal and one control.

More recently, Hubbard [9] has published his results in a few experiments upon splenectomized guinea-pigs. But here again the numbers are too small to be of much value. While numerous other investigators [10] have studied the relation of the spleen to resistance to infection, it can not be said that any of them have furnished much positive proof.

The blood changes following splenectomy, which might throw some light on the question of the animal's power to resist infection, have been the subject of considerable research.[11] These changes in dogs after splenectomy may be summarized as follows:

1. Moderate secondary anæmia, lasting one or more months.

2. Leucocytosis, most marked twenty-four hours after operation, and lasting for several months.

3. Increase in the polymorphonuclears and lymphocytes, and decrease in the mononuclears and transitionals, followed later by the reverse.

4. Absence of eosinophiles from the third to the eleventh week, followed by pronounced eosinophilia.

5. Appearance of nuclear particles in red blood-cells and presence of normoblasts in the circulating blood.

On the whole, they can not be said to furnish positive evidence of an impaired ability on the part of the body to resist infection; but it seems possible that the anæmia and leucocytosis which are temporarily observed are an indication of increased susceptibility to infection and imperfect reaction against it.

There are certain observations which lend color to the view that the spleen bears a special relation to infections in general. The prompt formation of an acute splenic tumor in many severe systemic infections indicates that the spleen is extremely sensitive to bacterial invasion. Moreover, if the myeloid metaplasia so frequently met with in the spleens of those suffering from generalized infection [12] indicates an effort at increased production of new blood-cells, this activity might be considered as only part of the process of resistance to the bacterial invader.

Whether the spleen really does pour out new blood-cells in considerable quantity has been the subject of considerable discussion, and the question is yet far from settled; but there is strong evidence that blood-formation is one of the functions of the spleen.[13]

While the spleen has been fairly definitely shown to be an important, if not the chief organ for the formation of bactericidal substances, it has been denied that it is greatly concerned with the production of agglutinins, precipitins, or antitoxins. This subject is, however, still *sub judice,* and the recent work of Hektoen [14] points strongly to the spleen and other lymphatic tissues as being concerned in the production of lysins, agglutinins, opsonins, and precipitins. Most of the experiments done in this field depend upon the reactions of tissue extracts outside the body, and, therefore, do not closely approximate the actual conditions in the living organism.

In order to arrive at some definite conclusion as to the importance of the spleen in opposing infection, the following series of experiments was begun on a large scale in the hope of definitely settling the question whether or not the removal of the spleen lowered an animal's resistance.

For these experiments, white and brindle rats were chosen, because they could be obtained readily in sufficient quantity for tangible results, and because in these animals the operation of splenectomy is very simple and is rarely followed by any complications such as wound infection, hemorrhage, or hernia. Furthermore, rats are particularly insusceptible to nearly all the common pathogenic organisms and unlikely to develop complicating infections which would confuse the results. While the rat is prone to be infested with a number of parasitic worms, the principal mortality results from septicæmia caused by an organism of the typhoid group which is very similar in its behavior to that causing hog and chicken cholera. This organism, the bacillus of rat plague, which has probably been described by a number of observers under different names, was isolated by Issatchenko,[15] and shown to be responsible for the rat plague then existing in St. Petersburg. While this organism has by some [16] been regarded as identical with the Ratten bacillus, the B. Danysz, and the B. enteritidis (Gaertner), it is unlike the last in being non-pathogenic to rabbits and in the fact that its broth cultures do not yield a toxic filtrate. The toxicity of the cultures, moreover, is destroyed by heating to 70° C. In this respect, as well as in its morphological and cultural characteristics, it resembles closely the B. typhi murium of Loeffler,[17] but as the latter is not ordinarily pathogenic for white rats, it seems safer to refer to the one employed by us as the B. Issatchenko or the bacillus of rat plague.

The organism is a slender motile rod which varies greatly in length in individual cultures and also according to the media used; short forms predominate. It is gram-negative, and takes the polar stain; in unstained preparations the translucent centre and darker poles are very striking.

The surface colonies on agar are round or oval, grayish, translucent discs. Gelatin is not liquefied. The broth cultures show general turbidity and pellicle formation followed later by sediment. Growth on potato is slow and scant, but after forty-eight hours a thin grayish covering appears. Litmus milk is turned first red, then blue-red. Glucose, galactose, levulose, and mannite are fermented, but not lactose, saccharose, dextrin, or maltose. There is no indol production. Growth takes place on all ordinary media.

We have found the infection to be both endemic and epidemic in the rats of this laboratory, and to cause nine-tenths of the mortality among these animals. Its behavior has been very carefully studied, and because of its morphology and growth characteristics, as well as because of the very uniform pathological lesions which result from its inoculation into an animal, it can with reasonable certainty be identified as the cause of death. Its cultures can readily be standardized; and as it can easily be recovered from the heart's blood after death, the organism proved to be most suitable for our experiments. These were as follows:

Thirty-six apparently healthy young brindle rats, weighing from 50 to 100 grammes each, were splenectomized under ether anæsthesia. Great care was taken to guard against any operative complications, such as hemorrhage or infection, and the subsequent autopsies showed that none had occurred.

A similar number of control rats of like weight and breed were subjected to a laparotomy in which one testicle was removed. As the testicle is larger than the spleen and the blood-vessels entering it are about equal in size to the splenic vessels, its transabdominal removal furnishes a very fair basis of comparison as to the effect of the operative trauma alone upon the subsequent health of the animals.

Both sets of animals were found to be active and thriving on the day following the operation. They were then exposed to chance laboratory contagion and kept under observation for several months. Whenever an animal in either series died, an animal of the other group was killed, and both were completely autopsied. Microscopical sections were made from all important organs, and cultures were taken from the heart's blood and from the peritoneal cavity or lung.

It was then observed that the splenectomized animals almost invariably died before the controls, and that the death-rate among them was 80.5 per cent. as compared with 38.9 per cent. in the control rats. Necropsy showed the following lesions: Congestion and parenchymatous degeneration of nearly all the important organs, varying from injection of the vessels and slight cloudy swelling to focal necroses or extensive disintegration of the parenchyma. If the animal had survived long enough for a reparative process to set in, there was replacement of the degenerated areas by new-formed granulation tissue.

These changes, which are characteristic of the tissue injuries wrought

by the bacillus of rat plague and certain kindred organisms, may be summarized as follows:

1. Lungs: congestion; *lobular pneumonia;* lobar pneumonia.

2. Thymus: parenchymatous degeneration; *necrosis;* and *replacement of the organ by granulation tissue.*

3. Heart: *myocardial degeneration* (fatty, hyaline), associated often with an interstitial cellular infiltration.

4. Liver: congestion; cloudy swelling; fatty infiltration; *focal necrosis* or extensive degeneration of essential cells with accumulation of fat.

5. Kidney: congestion; *parenchymatous degeneration* with *focal necrosis;* glomerular and tubular nephritis.

6. Pancreas: œdema and degeneration of parenchyma.

7. Lymph-nodes and lymph-follicles of intestine: acute lymphadenitis and focal necrosis.

8. Spleen: acute splenic tumor with focal necrosis.

These changes were found nearly always in the animals that died spontaneously, whereas in those that were killed as controls they were generally absent.

The peritoneal cultures were uniformly negative; but the heart's blood of the animals that died showed, as a rule, the presence of an organism of the typhoid group, and usually this could be identified as the bacillus of rat plague. The cultures from the animals killed as controls quite regularly remained sterile.

While it is probable that in the presence of an epidemic all the rats were exposed to infection with the bacillus of rat plague, and the autopsy findings suggest that many developed it, we conclude from the results (1) that the splenectomized rats were more prone to contract the disease; and (2) that, having contracted it, they showed less resistance to its ravages. This was evidenced by the greater frequency of septicæmia, by the graver character of the lesions observed *post mortem,* and by the high and rapid mortality in the splenectomized animals.

Since the mortality in the splenectomized animals was over twice that in the orchidectomized, it is probable that the lowered resistance of the spleenless animals to chance infection by the bacillus of rat plague was due to the removal of the spleen.

In order to test this out more fully a second series was begun. Seventy-two brindle or white rats of about 50 to 100 grammes' weight were splenectomized, and a like number of control animals castrated by the abdominal route. In this series, both sets of animals were exposed to chance laboratory contagion, and a record of the mortality in each group was kept, none being killed. After two months the records were compared. In the orchidectomized group, 21 rats, or 29.2 per cent., had died. The organs of most of these showed the lesions characteristic of rat plague infection, and this organism was cultured from the heart's blood. This is about the same mortality as that prevailing among stock labora-

tory rats that have not undergone operation. In the splenectomized group, 61 rats, or 84.7 per cent., had died, and these likewise showed the lesions of rat plague infection. The heart's blood was positive for this organism in about 50 per cent.

The results in this group three weeks after operation were as follows: 36 of the splenectomized rats had died of the prevailing epidemic; while but 7 of the castrated controls had died. The mortality was thus five times as great in the splenectomized group as in the controls. In each group, septicæmia was demonstrated by blood cultures as the cause of death in a high percentage. The organism was almost invariably that described as responsible for the epidemic or one morphologically akin.

These results showed that under ordinary laboratory conditions splenectomized rats were far less resistant to a common infection than those animals which had not been deprived of their spleens. The spleen then must in some way help to protect the animals against infection, since removal of another organ of equal size and weight by a similar operative procedure caused no impairment of the defensive mechanism.

To render the experiment still more conclusive, in a third series, two groups of young rats, comprising 88 animals in each, immediately following splenectomy in the one group and orchidectomy in the other, were injected subcutaneously with a sublethal dose of a broth culture of the bacillus of rat plague. The effect of this should be to hasten the death of those animals that were susceptible to rat plague, whereas those that had a certain amount of natural immunity would remain in good health and acquire an added resistance.

It was found that 1 c.c. of a mixed three- and five-day broth culture of the bacillus of rat plague was the least dose that could be relied upon to kill rats of 50 to 100 grammes' weight in five to ten days. In this experiment, therefore, 0.2 c.c. of a mixed three- and five-day broth culture was used. This dose produced in the castrated rats 22.7 per cent. of deaths, which is but little or no increase over the normal death-rate of non-operated, uninjected animals. In the splenectomized rats, however, the death-rate was enormous, amounting to 87.5 per cent. The date of death was likewise hastened, so that the majority of these animals died on an average of nine days following splenectomy. All showed the lesions characteristic of rat plague infection, and the organism was obtained in pure culture from the heart's blood in 82.9 per cent. of the cases, showing that death was undoubtedly due to bacteræmia caused by the inoculated organism.

In order to ascertain whether older rats were affected by splenectomy to the same degree, a fourth series was begun, in which 24 rats weighing from 200 to 250 grammes were splenectomized and a corresponding number castrated. Following operation each set received a subcutaneous inoculation of 0.6 c.c. of the mixed three- and five-day cultures of rat plague bacillus. The results in this series corresponded in every respect with

those in the previous lot, but were even more striking. Of the 24 adult rats which were splenectomized and injected with the culture of rat plague bacillus, 21, or 87.5 per cent., died within twelve days, and positive blood cultures were obtained in 90.5 per cent. Of the 24 castrated animals, however, only 3, or 12.5 per cent., died in the two months following operation. These all gave positive blood cultures. It will thus be seen that in this series the death-rate among the splenectomized animals was over seven times that in the orchidectomized group.

CONCLUSIONS

These results show in a very definite manner that while these animals may get along fairly well without the spleen in the absence of any infection, the reverse is the case when the organism is put to the strain of resisting acute bacterial invasion. Under the circumstances, we must infer that the spleen normally aids tremendously in resisting infectious processes in rats, and that its removal temporarily robs the body of its resistance until such a time, at least, as compensatory processes will have had a chance to reëstablish this.

The surgical bearing of these results is obvious. If, as we may reasonably infer, the physiological processes of mammals are similar, it is not improbable that the human body deprived of its spleen shows a similar increased susceptibility to infection. Bearing this in mind, some of the fatalities following splenectomy, especially where death was attributed to infection, may find a ready explanation and tend to increase our caution in the removal of this organ.

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